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THE SEASONAL VARIATION OF THE PHENOLIC DILACTONE LEUCODRIN IN *LEUCADENDRON ARGENTEUM* R. Br.

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ABSTRACT

The seasonal variation of the occurrence of leucodrin in *L. argenteum* was followed for 15 months; samples consisting of 10 g twigs were collected for testing every three weeks. The leucodrin was extracted from the plant material and partially purified before the concentration of its TMS ether was determined by GLC. The results show that the leucodrin content of the leaves increased as the new leaves of the terminal bud increased in size. This increase was from approximately 9 mg to 15 mg/g fresh weight. After the leaves were fully developed the leucodrin concentration decreased until the next season when new leaves started to develop from the terminal bud. This same pattern has been reported for other phenolic compounds from different plant sources. The moisture content of *L. argenteum* leaves also showed a seasonal variation; the maximum moisture content was found during the wet summer with minimum moisture content being found during the dry winter when there was no rain for several months.

UITTREKSEL

DIE SEISOENSVERANDERING VAN DIE FENOLIESE DILAKTOON LEUCODRIN IN *LEUCADENDRON ARGENTEUM* R. Br.

Die seisoensverandering van die voorkoms van leucodrin in *L. argenteum* was vir 15 maande gereeld ondersoek; monsters van ongeveer 10 g stingels was elke drie weke versamel en dan getoets. Die leucodrin was uit die plant materiaal ge-ekstraheer en gedeeltelik gesuiwer voordat sy eter bepaal is deur gaschromatografie. Die resultate bewys dat die leucodrininhoud van die blare algaande vermeerder soos die nuwe blare van die terminale bloeisel groter word. Hierdie vermeerdering was van ongeveer 9 mg tot 15 mg per g vars gewig. Nadat die blare ten volle ontwikkel was het die leucodrinkonsentrasie verminder tot die volgende seisoen wanneer die blare weer begin ontwikkel het vanaf die terminale bloeisel. Dieselfde patroon was gerapporteer vir ander fenoliese stowwe van verskillende ander plantbronne. Die voggehalte van *L. argenteum* blare het ook 'n seisoensvariasie getoon; die maksimum voggehalte is gevind gedurende die nat somer en die minimum voggehalte gedurende die droë winter wanneer daar geen reën was vir 'n hele paar maande nie.

INTRODUCTION

Leucodrin has been known to occur in various *Leucadendron* spp. since 1886 and the structure of this phenolic dilactone has now been fully established (Perold and Pachler, 1966). Its distribution includes the genus *Leucadendron* and several *Leucospermum* spp. (both in the Proteaceae) and it has been found in varying amounts in the different species examined. Murray (1962) reported 15% and

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Murray and Bradshaw (1966) reported up to 20 % leucodrin in the leaves of *Leucadendron salignum* while Plouvier (1964) reported 2,3 % in the influorescences of *Leucospermum reflexum* and 1,1 % in its leaves. He also found similar results for *Leucadendron argenteum*.

It has been suggested that leucodrin could act as a carbohydrate storage product (Hegnauer, 1969). He based this decision on the large amounts of the compound present and its relationship to sugar. One way of incorporating the sugar may involve a Michael-type condensation of *p*-coumaric acid and L-γ-galactonolactone (Diamand and Rogers, 1964; Perold, Hodgkinson, Howard and Kruger, 1972). Another possible biosynthetic route could involve the reductive C-C coupling of *p*-coumaric acid and ascorbic acid (Couchman *et al.*, 1973). Whichever way leucodrin is biosynthesized, one molecule of carbohydrate is combined with one molecule of *p*-coumaric acid which itself is formed from the carbohydrate pool via the shikimic acid pathway.

Since equal amounts of phenolic and sugar are involved in this unusual compound it was decided to attempt to determine which way leucodrin was behaving: as a phenolic or as a carbohydrate. One way to solve the problem was to study its variation in concentration during seasonal change. The literature reports seasonal changes for a wide variety of plant constituents and the results of this study could then be compared with these reports.

Over the past few years much has been published about biological rhythms; there are many variations in the type of rhythm, perhaps the best known being the 24 hour or circadian period. Some work has been published on annual variation in organisms and it has been found that for the various metabolic pools and functions, maxima usually occur mid-summer with the minima occurring in the winter. Studies using beans show that O_2 consumption and water uptake followed the same well-defined pattern of maximum rate in summer and minimum in winter. Similar studies on O_2 consumption in potatoes showed the same pattern (Palmer, 1976). Also, the plant to be sampled in these experiments was growing in a summer rainfall area and it was decided to monitor its leaf moisture content. It could be of interest to see if the leaf moisture content follows the pattern of rainfall, i.e., very high rainfall in the summer with no rain for several months during the winter.

MATERIAL AND METHODS

All plant material was collected from one tree which was located near Jan Smuts House, University of the Witwatersrand, Johannesburg. Samples were collected at the same time of day every 3 weeks for 15 months, starting in February, 1977. The moisture determinations were done in triplicate by drying the samples in an oven at 120 ° for 20 hours. Samples, taken for both moisture and leucodrin determinations, were the tips of branches and weighed approximately 10 g.

The leucodrin content was determined by GLC and the samples were prepared in the following manner. The sample of fresh plant material taken for the leucodrin determinations was ground in MeOH in a Waring Blendor and then exhaustively extracted in a Soxhlet extractor. This extract was partially purified by column chromatography on silica gel using benzene:ethyl acetate:methanol (5:3:2 v/v). The fractions containing leucodrin were collected and made to a 10% solution in MeOH, and 0,1 mℓ of this was taken for determination by GLC. All determinations were done in duplicate with standard leucodrin solutions of known concentrations run as references. All samples were taken to dryness in small tubes which were then sealed with septa prior to silylation. The trimethylsilylethers of the samples were formed by silylation using 50 $\mu\ell$ of a stock solution which comprised 10 mℓ dry pyridine, 1 mℓ trimethylsilylchloride, and 2 mℓ hexamethyldisilazane. After heating at 90° for 1 hour, 1 $\mu\ell$ of the silylated sample was injected into a Pye series 105 GLC apparatus. The column was 10% Si 52 on Anakrom and had dimensions of 0,004 × 2,0 m. All samples were run isothermally at 300 °C.

The leucodrin content was determined only on a fresh weight basis and the data in Figure 1 for the leucodrin content based on dry weight was calculated using the moisture content.

RESULTS AND DISCUSSION

The leucodrin content of *L. argenteum* as it varies with seasons is presented in Figure 1; the leucodrin content is presented on both a dry weight basis and a fresh weight basis. This is done to allow for any variation in moisture content which also shows a seasonal variation. Figure 1 shows that this seasonal variation in moisture affects the pattern of leucodrin content. On a fresh weight basis the maximum leucodrin content appears to maintain a plateau from July until November which covers the end of winter through spring until the beginning of summer. When the leucodrin content is calculated on a dry weight basis it follows a pattern having a maximum during the period of leaf expansion in late winter and early summer and declining during late summer which is the period of negligible growth.

Figure 2 reports the seasonal variation of leaf moisture content along with the actual rainfall recorded at Joubert Park, Johannesburg; this weather station is approximately 1 km from the point where the plant material was collected. From this figure it can be seen that the maximum moisture content of the leaves of *L. argenteum* was found during the rainfall season, summer, and the minimum leaf moisture content occurred during the winter when there was no rain at all for several months.

It is probable that Plouvier (1964) found leucodrin present in *Leucodendron argenteum* but not in *Leucospermum reflexum* as he reported. The compound found in the latter species was probably the diastereoisomer, conocarpin, or one

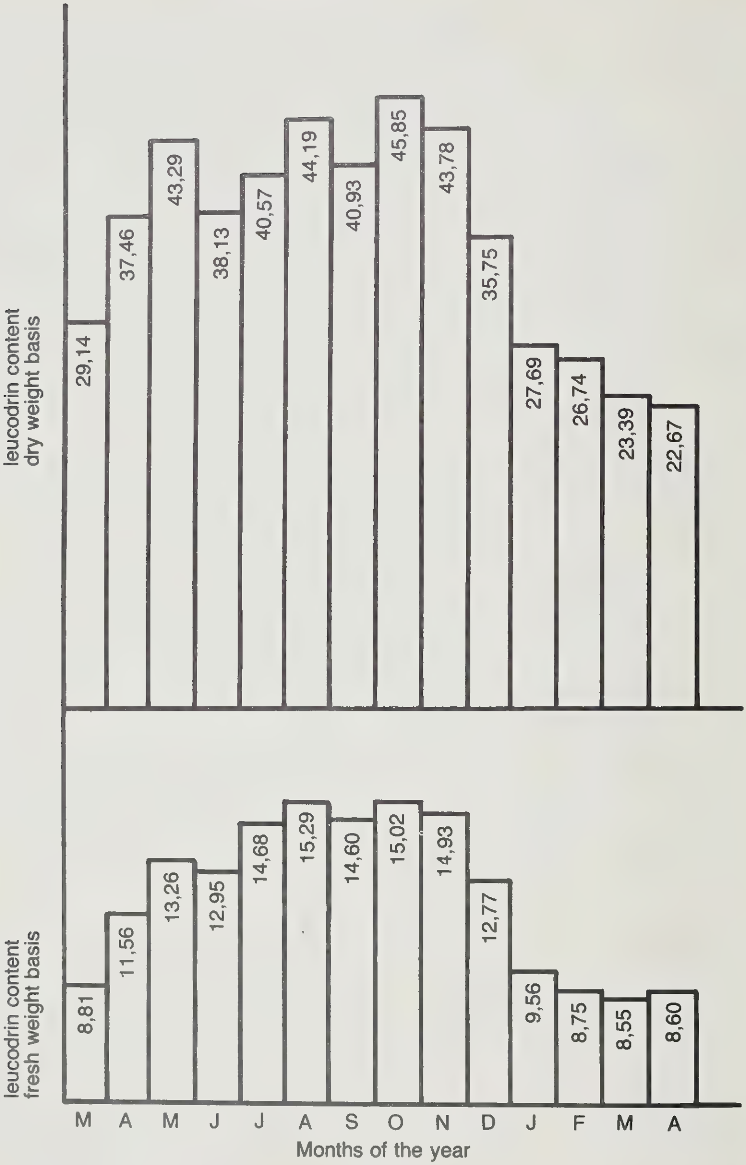

FIG. 1.
Leucodrin content of *Leucodendron argenteum* leaves in mg/g dry weight and mg/g fresh weight.

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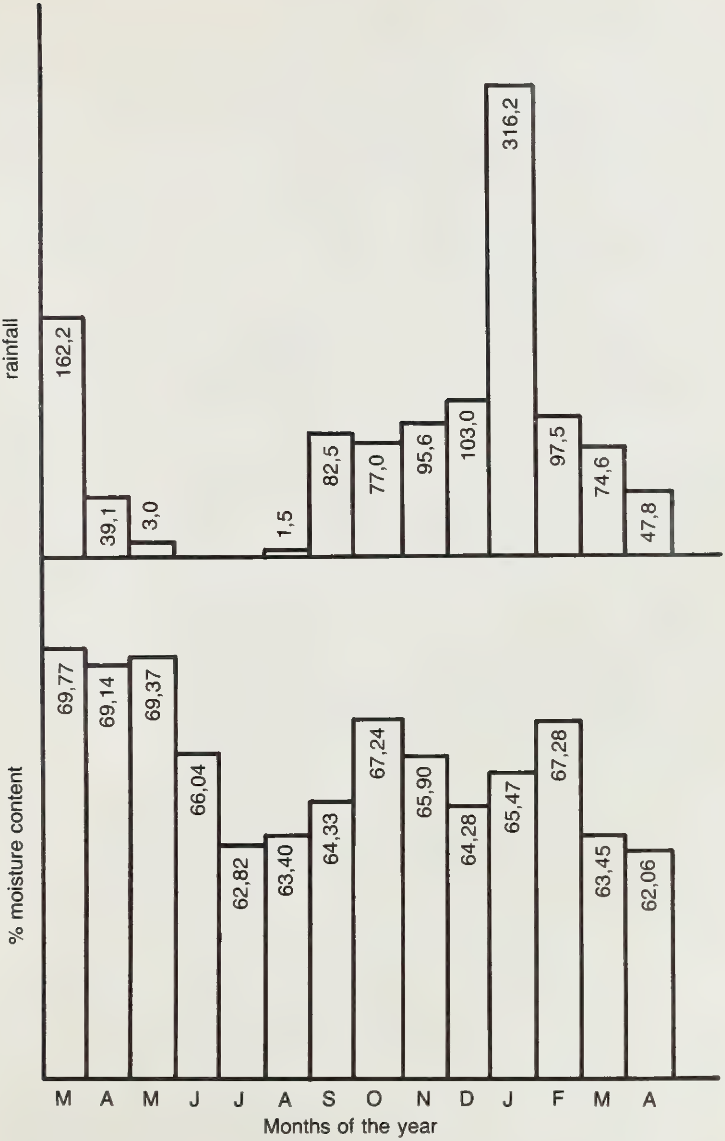

FIG. 2.
Leaf moisture content of *Leucadendron argenteum* and the rainfall amount (mm) for the same fourteen month period.

of its related compounds: reflexin or conocarpic acid (Kruger and Perold, 1970; Perold, Hodgkinson and Howard, 1972). It has been found that all *Leucadendron* spp. and a few *Leucospermum* spp. examined contained leucodrin while the majority of *Leucospermum* examined contained the diastereoisomer, conocarpin.

During this study *Leucadendron salignum* was used as a source of leucodrin for one quantitative determination. The reason only one determination was carried out was due to the local scarcity of plant material of this species and its relatively small leaf size. It was found that *L. salignum* leaf material yielded 5,40 % leucodrin on a fresh weight basis and 11,25 % on a dry weight basis. This is in comparison with the findings of Murray and Bradshaw (1966) who reported up to 20 % leucodrin for this species.

Some work has been reported on the changes in plant hormone concentrations in leaf material during the growing season. Van Staden (1976, 1977) found that in both *Ginko biloba* and *Salix babylonica*, the cytokinin activity was related to leaf maturity. In young, immature leaves the cytokinin activity was extremely low but this increased as the leaves matured. In terms of season the lowest cytokinin content was found during October and the highest content was in summer through autumn (December until June). Another study on the changes in cytokinin content was carried out on *Populus* X *robusta* (Hewett and Wareing, 1973). This study showed that cytokinin activity was at a maximum in expanding leaves, and falling off as the leaves aged. This gave the highest concentrations of cytokinins in mid-summer.

The trees used in these above studies were deciduous while *L. argenteum* is an evergreen broad-leafed tree. Like the cytokinins, leucodrin increased during leaf development but it is probable that *L. argenteum* produces its leaves earlier in the season than those used in the cytokinin activity studies and hence the maxima and minima for concentration do not coincide.

Fry and Phillips (1977), in their study on photosynthesis in conifers in relation to annual growth cycles, found a similar pattern to leucodrin production. Using this evergreen species they found that photosynthetic efficiency of new needles showed an overall decline from just after needle maturity until just before bud-break in the next season. After bud-break the photosynthetic rate increased parallel with needle development until the previous maximum was reached with leaf maturity falling off to a minimum before the terminal bud breaks is very similar to the leucodrin concentration in *L. argenteum*.

To assess the carbohydrate production pattern of plants, sugar cane was taken as an example. Production of sugar was lowest at temperatures below 21 °C, and highest along with maximum growth rate, during the hot, sunny days of summer (above 32 °) (Bass *et al*., 1957). The pattern of leucodrin accumulation in *L. argenteum* certainly is not similar to that of sugar in sugar cane. Comparing Figure 1 and Figure 3 it can be seen that during the hottest days of summer (October to March) the leucodrin content is decreasing instead of increasing as sugar does.

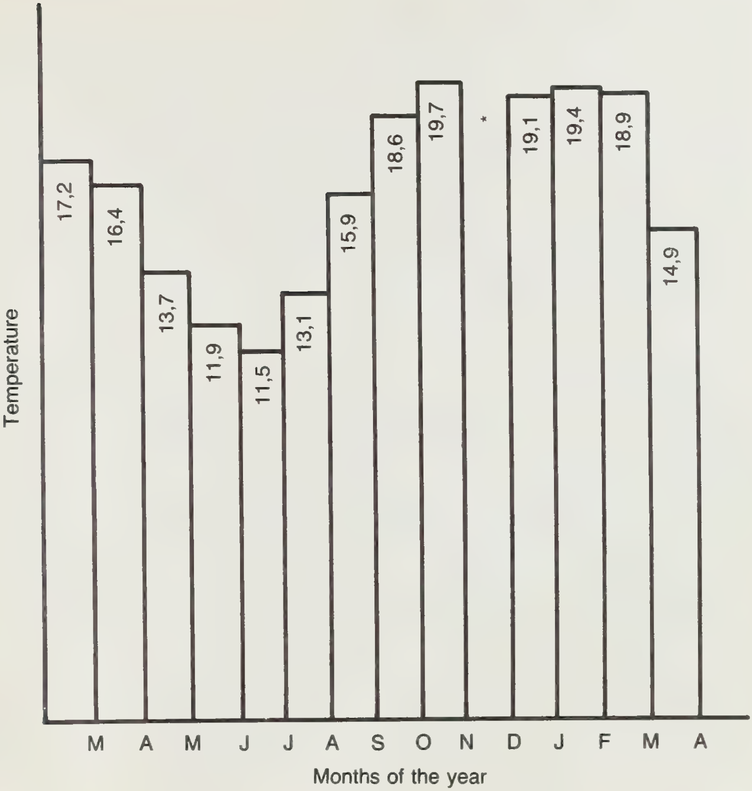

FIG. 3.
Mean monthly temperatures (°C) at Joubert Park, Johannesburg for the experimental period.
*Data not available.

Phenolic compounds exhibit a wide variation in concentration due to season. A linear increase in the amount of shikimic acid was found with the increase in weight of the leaves of *Eucalyptus sieberiana* (Hillis, 1958). The maximum amount of shikimic acid was present in the leaves which had just reached maximum size. After this the shikimic acid content of fully developed leaves decreased with the age of the leaf. Hillis and Swain (1959) found a very similar pattern in *Prunus domestica*. They analysed leaf material for total phenols, leucoanthocyanidins and flavonols, at intervals during the growing season. The results showed that the amounts of these compounds increased with increasing leaf size until the leaves reached maximum size and then the content of phenols decreased as the leaves aged.

The concentration pattern of the phenolic dilactone leucodrin follows very closely the concentration patterns of shikimic acid or total phenols. In *L. argenteum* the terminal bud is formed at the end of one season but due to the mild winter it does not lie dormant during the winter but rather maintains a slow growth pattern maturing in the early summer. It is this same pattern that the leucodrin concentration follows, reaching a maximum in the newly developed mature leaves, and decreasing as the fully developed leaves age until the next season's terminal bud starts developing. Since shikimic acid is a precursor of *p*-coumaric acid which, in turn, is probably a precursor of leucodrin, then the leucodrin content should follow the same pattern as the shikimic acid content.

Why does leucodrin accumulate to such a large extent in the leaves of *Leucadendron* spp.? Since its seasonal variation in concentration closely follows the seasonal pattern of a phenolic compound and not a carbohydrate, it is probably functioning as a phenolic type compound and not, as Hegnauer suggested, a carbohydrate. Since many phenolic compounds are phytotoxic, leucodrin formation could simply be one method of detoxifying *p*-coumaric acid. It has been shown that some plants detoxify hydroxylated cinnamic acids by forming glucose esters (Asen and Emsweller, 1962; Glennie and Bohm, 1966). This addition of a sugar onto the phenolic compound could also aid the plant in translocating the compound or make it easier for transport across membranes, e.g., transporting it into the vacuole for storage.

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